

# A High Sensitive LC-MS/MS Method for Quantitation of Formoterol in Human Plasma

Dawei Zhou, Wenzhong Liang, Xiping Fang, and Jinn Wu  
XenoBiotic Laboratories, Inc., 107 Morgan Lane, Plainsboro, NJ 08536

**XenoBiotic**  
Laboratories, Inc.

## Overview

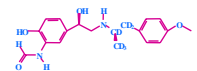
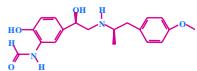
A highly sensitive and specific liquid chromatography- mass spectrometry (LC-MS/MS) method capable of quantifying formoterol in human plasma is described.

In this method, the drug was extracted from a 0.5 mL sample of plasma using a strong cation exchange solid phase extraction method. Separation was performed on a reverse phase C8 column. Detection was achieved using an AB/SCIEX API-4000 tandem mass spectrometer employing turbo-ion spray ionization in the positive ion mode along with multiple reaction monitoring (MRM). The lower limit of quantitation was 0.4 pg/mL.

## Introduction

In a recent study for obstructive airway disease, including asthma and COPD, formoterol exhibited a rapid onset of action comparable to the short-acting bronchodilator, VENTOLIN<sup>®</sup>, as well as a duration of action of up to 24 hours. Previous bioanalytical methods have been developed for 1-2 mL sample volumes, which would necessitate large individual SPE cartridges making the sample preparation time longer, and also require the collection of higher total volumes of plasma. The current method was developed to reduce the sample preparation time, lower the detection limit and lower the volume of plasma required. We report a rapid, specific, and highly sensitive LC-MS/MS method capable of quantifying formoterol at levels as low as 0.4 pg/mL from 0.5 mL of human plasma.

## Structures



## Experimental

### Sample Preparation

Formoterol and the internal standard (formoterol-D<sub>6</sub>) were transferred onto the preconditioned Strata X-C Polymer Strong Cation 96-well plate (Phenomenex). The plates were preconditioned with 1 mL of methanol and 1 mL of 0.1% formic acid aqueous solution. The SPE plates were then washed with 2 mL of 0.1% formic acid solution, 0.8 mL of acetonitrile, and 0.8 mL of a mixed solution of dichloromethane and isopropyl alcohol. Both analyte and internal standard were then eluted using a mixed solution of dichloromethane and isopropyl alcohol containing 2% of ammonia.

The eluted samples were evaporated to dryness for approximately 30 min in a 96-Well Nitrogen Evaporator (EvapArray<sup>™</sup>) at approx. 35 °C. The residue was dissolved in 125  $\mu$ L of 100 mM ammonium formate in water for analysis.

### Liquid Chromatography:

HPLC System Shimadzu LC-10AD  
Analytical Column: C8 column, 2.1 x 50 mm, 3  $\mu$ m  
Mobile Phase A) 10 mM ammonium Acetate in water (pH4)  
Mobile Phase B) Methanol  
Gradient  
Flow rate: 0.5 mL/min  
Injection Volume: 25  $\mu$ L

### Mass Spectrometry

MS System: AB Sciex API-4000  
Condition: LC(+)-ESI-MS/MS (MRM)  
MRM Transition:  
Formoterol: 345.2  $\rightarrow$  149.1  
Formoterol-D<sub>6</sub>: 351.3  $\rightarrow$  155.1

## Results and Discussion

Table I. Validation Data Summary

Calibration Range		0.4 to 100 pg/mL	
Accuracy & Precision	QC Conc. (pg/mL)	Accuracy RE%	Precision CV%
	Inter-Batch (n=18)	Low 0.4	2.00 13.57
	Low 1	5.00 8.94	
	Medium 40	9.00 3.81	
High 90	8.78 3.49		
Method Recovery		Compared with Nominal Value (%)	
Long-Term Storage Stability		> 71.95	
Freeze/Thaw		Condition	Accuracy RE%
Bench-Top		3 Cycles, < -70 °C	< -9.89
Autosampler Extract Stability		4 hrs, Room Temperature	< 11.1
Long-Term Storage Stability		4 Days, Room Temperature	< 9.75
		117 Days, < -70 °C	< 5.30

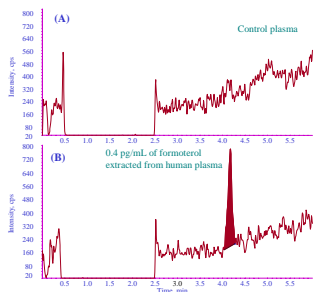


Figure 1. Ion chromatograms of blank plasma (A), and 0.4 pg/mL formoterol extracted from plasma (B)

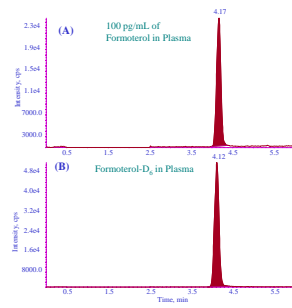


Figure 2. Ion chromatograms of 100 pg/mL formoterol extracted from plasma (A), and the internal standard extracted from plasma (B)

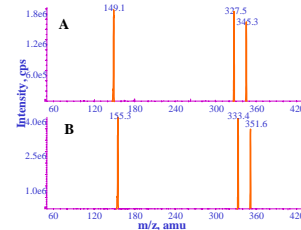


Figure 3. LC(+)-ESI-MS/MS product ion spectra of Formoterol (A) and Formoterol-D<sub>6</sub> (B)

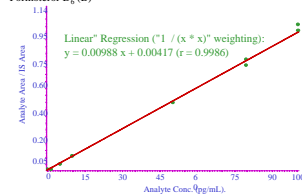


Figure 4. Representative calibration curve for the determination of formoterol in the range from 0.4 pg/mL to 100 pg/mL in human plasma

- In previous methods larger plasma sample volumes (1-2 mL) were needed in order to achieve a lower detection limit. These sample volumes would make preparation much longer requiring large individual SPE cartridges and introduce more error in the preparation procedure. The current method employed special SPE elution solution by which the extraction method recovery was dramatically increased. Therefore, only 0.5 mL of human plasma was required to reach the requisite sensitivity.

- A 0.5 mL sample volume afforded the use of a 96-well SPE plate format, instead of individual cartridges. In addition, the TOMTEC liquid handling system made sample preparation quick and consistent.

- Excellent linearity was obtained with a correlation coefficient greater than 0.9936. The inter-day precision (CV%) and accuracy (RE%) for all QC plasma samples, including LLOQ were  $\leq$ 13.6% and  $\leq$ 9%, respectively (Table D). Three freeze/thaw cycles, ambient temperature storage for up to 4 h prior to analysis, and three month long-term storage at -20°C appeared to have little effect on the quantitation.

## Conclusions

A rapid and specific LC-MS/MS method was developed and validated for quantifying formoterol with a lower limit of quantitation of 0.4 pg/mL from a 0.5 mL of human plasma sample.